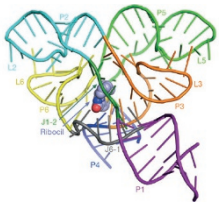
RIBOSWITCHES

A bacterial antivitamin

Nature **526**, 672–677 (2015)

NATURE



Antimicrobial strategies frequently target bacterial pathways that follow distinct routes from those in the human host or that humans lack entirely. Riboswitches—small noncoding RNA motifs that regulate gene expression in response to the specific binding of a small-molecule metabolite—are viewed as an attractive but underexplored class of antibacterial targets. Early efforts focused on the flavin mononucleotide (FMN) riboswitch, which regulates genes controlling the biosynthesis and transport of the essential vitamin riboflavin, have so far resulted in compounds with limited target selectivity. Howe *et al.* now report the identification of a specific inhibitor of the FMN riboswitch that displays potent antibacterial activity. The authors demonstrated that riboflavin biosynthesis genes such as *ribA* and *ribB* are conditionally essential in *Escherichia coli*, a property that facilitated the phenotypic screening of ~57,000 growth-inhibitory compounds seeking agents with *E. coli* bioactivity that could also be suppressed by exogenous riboflavin. The screen identified ribocil, a synthetic compound with a chemical scaffold entirely distinct from that of riboflavin, which specifically inhibited the production of riboflavin and its metabolites in treated cells. To identify the potential target of ribocil, the authors selected compound-resistant *E. coli* strains and used whole-genome sequencing to show that all resistance mutations exclusively map to the FMN riboswitch that controls *ribB* expression. The FMN riboswitch was further established as ribocil's target through binding measurements, showing it to be a competitive inhibitor of FMN, and reporter gene assays, demonstrating that ribocil inhibited GFP expression under the control of wild-type, but not mutated, FMN riboswitch constructs. A crystal structure of (*S*)-ribocil in complex with the FMN riboswitch from *Fusobacterium nucleatum* confirmed that ribocil binds to the FMN pocket and suggested how resistance mutations may weaken ribocil binding. Finally, in a murine sepsis model, ribocil displays potent antibacterial activity that is dependent on its FMN-inhibitory properties. Beyond illustrating the utility of phenotypic screening and target identification in chemical biology, the study provides compelling evidence that bacterial riboswitches, and possibly other classes of noncoding RNA elements, may serve as new and as yet largely unexploited druggable targets. *TLS*

RNA MODIFICATION

Stressing the message

Nature **526**, 591–594 (2015)

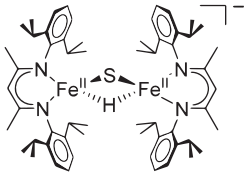
N^6-Methyladenosine (m6A) is the most abundant mRNA post-translational modification and is mediated by nuclear methyltransferase (MeT) proteins (including METTL3, METTl14 and WTAP). FTO is a demethylase that removes this modification. Once the modification occurs, m6A mRNA is specifically recognized in the cytoplasm by the RNA-binding protein YTHDF2, which regulates mRNA stability. In addition, m6A-modified mRNA regulates RNA-protein interactions and translational efficiency. However, it was not known whether this type of modification responds to environmental or cellular changes. To test this, Zhou *et al.* immunoprecipitated RNA with an m6A-specific antibody and used next-generation sequencing to examine changes in methylated RNA species in cells in the presence or absence of heat shock. Heat shock–upregulated genes, such as *HSPA1A*, exhibited an abundance of m6A, particularly in the 5′ UTR, whereas downregulated genes contained less methylation in the 5′ UTR. The authors found that heat-shock treatment did not alter the protein levels or activity of the nuclear MeT proteins but increased the transcription and nuclear translocation of YTHDF2. YTHDF2 knockdown decreased 5′ UTR m6A levels, indicating that YTHDF2 was necessary for the heat shock–induced m6A modification. YTHDF2 promoted m6A modification by effectively competing with FTO to prevent demethylation as knockdown of FTO increased 5′ UTR m6A levels in untreated conditions. Given that the 5′ UTR is involved in regulating mRNA translation, Zhou *et al.* performed ribosome profiling in heat shock–treated cells and noted that m6A 5′ UTR–modified mRNA exhibited increased ribosomal occupancy that was dependent on increased YTHDF2 levels. Because heat shock stress suppressed cap-dependent translation, the authors thought that the presence of m6A might act as a cap substitute to activate mRNA translation. Indeed, the addition of m6A to a 5′ UTR construct increased translational activity independent of the normal cap. Consistent with this hypothesis, mutation of the m6A site in Hsp70 mRNA or construction of an Hsp70 5′ UTR construct that failed to undergo m6A modification resulted in decreased translational efficiency. Overall, these findings reveal a role of m6A modification in providing a unique mode of mRNA translation. *GM*

METALS

A hydride out of hiding

J. Am. Chem. Soc. **137**, 13220–13223 (2015)

JACS



Nitrogenase uses an iron-sulfur cluster–based cofactor to convert dinitrogen into ammonia, but the full details of the mechanism have proven elusive. One topical question is whether hydride ions are part of the reaction mechanism, and recent experimental data does provide evidence for Fe-H bonds. However, there are no inorganic complexes of iron sulfides with hydride ligands, limiting the available knowledge about expected structures and raising questions as to the chemical feasibility of such an unusual intermediate. To enable further study, Arnet *et al.* now report the synthesis and analysis of such a model complex. The authors began with a known iron hydride complex, $[L^{Me}FeH]_2$, in which L^{Me} is a bulky bivalent ligand. A heated reaction with sodium dodecanethiolate followed by treatment with a metal chelator led to the replacement of one hydrogen atom with a sulfur atom, as demonstrated through X-ray diffraction analysis, Mössbauer analysis and mass spectrometry. With the complex in hand, the authors examined its reactivity. Nitrogenase is capable of reducing alkynes; however, the addition of an alkyne substrate to the chelated complex led to a bound alkyne product and loss of H_2 gas, leading the authors to speculate that the hydride is a better base than a nucleophile. How this translates to the nitrogenase mechanism, and whether steric effects unique to the ligand environment influenced the result, will require further study. Nitrogenase is known to reduce CO_2 to CO, CH_2O_2, or CH_4, but the initial reaction step is unknown; exposing the synthetic complex to CO_2 led to the formation of a CHO_2 ligand, suggesting that formate may be the first step along the pathway. Finally, the complex was capable of binding N_2 upon reduction. These results provide new opportunities to test and explore nitrogenase's function. *CG*

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